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(54) Title: SKIN PROTECTIVE COMPOSITIONS AND METHOD OF INHIBITING SKIN IRRITATION

(57) Abstract

A method for protecting the skin from contact with an allergenic agent or toxic agent comprising applying to the skin of a subject, prior to contact with said skin irritating allergenic agent and/or said toxic agent, a biocompatible, substantive, film-forming protective composition. The protective composition comprises at least one aminopolysaccharide and optionally a suitable carrier or base therefor. The film formed by the at least one aminopolysaccharide acts as a barrier to the allergenic and/or toxic agents to prevent or at least reduce their contact with the skin. The at least one aminopolysaccharide is preferably selected from the group consisting of chitosonium polymers and covalent chitosan derivatives. Preferably, the method further comprises applying an immobilization composition over the applied protective composition. The immobilization composition comprising at least one anionic compound, preferably an anionic polymer. The at least one anionic compound is present in an amount effective to render the aminopolysaccharide water-insoluble. Likewise, the immobilization composition preferably further comprises a suitable base or carrier therefor, which may be the same or different from that of the protective composition. The immobilization composition may further contain insect repellents and/or UV absorbers which may be toxic and/or skin irritating. However, the protective composition film prevents or minimizes their contact with the skin.

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SKIN PROTECTIVE COMPOSITIONS AND METHOD OF INHIBITING SKIN IRRITATION

BACKGROUND OF THE INVENTION

1. Field of the Invention

This invention relates to a skin protective composition for topical application to the skin to act as a barrier and prevent or minimize skin contact with natural and synthetic materials which are toxic and/or produce allergic contact dermatitis, most commonly poison ivy, poison oak or poison sumac, and a method for preventing or minimizing skin contact with materials which are toxic and/or produce allergic contact dermatitis.

2. Description of the Prior Art

Contact dermatitis manifests itself in an inflammation of the skin. In mild cases the symptoms are itching, burning or reddening of the skin. In more severe cases vesiculation and edema may be present and may be followed by weeping and crusting. The most severe cases may be accompanied by bleeding vesicles and gross edema.

Contact dermatitis can be classified as allergic contact dermatitis or as primary irritant dermatitis. Although the symptoms of both types of dermatitis are similar, there are some basic differences which are widely recognized.

Primary irritant dermatitis is the more common form of contact dermatitis and is caused by irritating agents which will cause dermatitis in all persons upon sufficient exposure. The period between contact with the primary irritant and the onset of symptoms is short or absent.

Allergic contact dermatitis may be caused by many substances which contact the skin. However, in this type of dermatitis a given substance may cause a dermatological reaction in certain subjects only. This reaction usually does not occur with the initial contact, but only upon subsequent exposures. Moreover, the reaction occurs only in

these now "sensitized" subjects and there is a time interval between contact and developing of symptoms. Sensitization is caused by previous contact to the irritating substance. Some persons never become sensitized, others require numerous contacts and some require only a few contacts for sensitization. According to Dr. William Epstein, as reported in the Smithsonian, Volume 16, Number 5, dated August, 1985 by Noel Vietmeyer:

> "Between 15 and 25% of us are essentially immune, 25% are mildly sensitive and don't normally develop severe reactions, 25 to 30% are moderately sensitive and break out significantly with the amount of urushiol found in one leaf and 10 to 20% are so exquisitely sensitive that less than one leaf produces intense dermatitis . . . . "

Thus, an essential difference is recognized between allergic contact dermatitis and primary irritant dermatitis, the latter being predictably induced by substances irritating to all persons and the former being erratically induced, if at all, and only after sensitization.

Among the most common naturally occurring allergens capable of sensitizing and causing allergic contact dermatitis in a large proportion of subjects are the antigenic plants of the genus Rhus, such as poison ivy, poison oak, and poison sumac. The active toxin thereof has long been known to belong to a group of organic compounds called urushiol. Urushiol has been identified as comprising a number of antigenic compounds. The antigens are 1,2-dihydroxy benzenes (catechols) with a 15 or 17 carbon aliphatic side chain in the 3 position which has varying degrees of unsaturation. It is not unusual for the sap of different plants to have mixtures of dihydroxy benzenes or to have these compounds in common but at different concentration levels.

The allergic contact dermatitis is reported in the literature to be caused by the catechol moiety as the primary allergen. The aliphatic hydrocarbon side group allows

bonding to and penetration of the skin. Thus, the oily substance urushiol, when in contact with the skin, penetrates the outer skin layers and begins to chemically bind to the skin cells. The body sees the combination of the urushiol in chemical combination with a skin cell as a foreign intruder. The immune system immediately rushes large white cells called macrophages and T-lymphocytes to destroy the affected skin cells. Dr. William Epstein explains, Ibid.:

> "It's the body's own over-reaction that causes the complications. In sensitized persons, the area fills up with the white blood cells and they release so much cell-destroying toxins that they tear apart even the skin itself. That's what produces the blisters and suppurating sores."

The symptoms of reddening of the skin, formation of an itchy rash and often blistering of the skin common to all types of contact dermatitis are particularly familiar to many as a result of contact with poison ivy or other plants of the genus Rhus. Such dermatologic reactions are extremely irritating and in severe cases can cause temporary incapacitation of an individual. With the advent of the greater use of the outdoors by people, such as is experienced in camping, hiking, nature study and similar activities, especially in wooded or relatively less populated areas, the probability of contact with skin irritating plants is increased and therefore the need for protection against irritation resulting therefrom becomes apparent.

Strangely, however, the allergen urushiol does not appear to affect animals and household pets. Cats and dogs can be exposed and actually play in the area without being affected, but can infect their owners by brushing up against their skin and transferring the urushiol on their coats to the unexposed areas of the human anatomy.

A major problem as to the contact with urushiol from poison oak, poison sumac and poison ivy is encountered by the foresters of the U.S. Forestry Service. This is particularly

severe in the case of forest fires, where the soot and gases from the burning flames contain urushiol, which can get onto the foresters fighting the fire and even into their respiratory system. This is further complicated by the fact that urushiol coats fomites, such as clothing, utensils, even carbon and soot in the area of forest fires and can therefore provide another method of contact, even outside the area of the plants themselves.

While allergic contact dermatitis due to poison ivy, poison oak, poison sumac and like antigenic plants is widely known, it is equally well established that allergic contact dermatitis is caused by a large number of materials encountered by workers in industry. Such materials may be end products, raw materials, intermediates and by products of industrial processes. The problem of occupational allergic contact dermatitis is a significant one resulting in lost man hours of production, lost wages, discomfort to workers, job changes and workmen's compensation payments. Examples of materials known to cause allergic contact dermatitis among industrial and non-industrial workers are dinitrochlorobenzene, phenols, benzoic acid and others, insecticides containing pyrethrum or Rotenone, dye intermediates such as aniline, nitro compounds, anthracene and derivatives thereof, benzidine and its compounds, naphthylamines and benzanthrone and its compounds, dyes such as paraphenylenediamine, aniline black, para-amido-phenol, amido-azo-toluene, amido-azo-benzene, Malachite Green, Metanil Yellow, Nigrosine and Rosaniline, photo developers such as hydroquinone, para-amido-phenol and pyrogallol, rubber accelerators and antioxidants such as hexamethylenetetramine, tetramethyl thiuram monosulfide, paratoluidine, phenyl beta-naphthylamine and triethyl trimethyl triamine, coal tar and its derivatives such as pyridine and phenanthrene, explosives such as trinitrotoluol, tetranitro-methyl aniline, ammonium nitrate and sodium

nitrate, industrial lubricants such as substituted phenols, e.g. CARDOLITE (Reg. TM) materials which are like urushiol less the hydroxyl group at the 2-position, and synthetic and natural resins such as wood rosin and phenol formaldehyde.

There is also a growing concern regarding the systemic absorption through the skin of materials which are toxic and/or allergenic and which are presently in common use as insect repellents such as DEET (N,N-diethyl-m-toluamide) and UV absorbers or sunscreens such as benzophenone, PABA (para-aminobenzoic acid) and esters thereof and substituted PABA, esters of para-methoxycinnamic acid, benzotriazoles and aminophenols. There is added concern regarding sunscreen products having high SPF ratings. These materials require higher contents of the sunscreen active(s) to achieve these ratings. Several sunscreen actives are known to be irritating and even carcinogenic at higher doses.

It is known that protection against skin irritation can be achieved in subjects sensitized to allergens such as plants of the genus Rhus by exposing the subject to a controlled series of contacts with the allergenic irritant. See U.S. Pat. No. 4,428,965 to Elsohly et al. and references cited therein. The concentration of allergen, usually in an innocuous vehicle, in each subsequent controlled exposure can be increased so as to build-up a degree of immunity to the allergenic substance. Such a procedure results in temporary or partial protection against skin irritation caused by contact with the specific allergen or closely related allergens. However, the procedure, carried out under a physician's direction, is somewhat tedious, inconvenient and often uncomfortable.

Many folk remedies have been proposed for use after contact with urushiol. These include morphine (topically), bromine, kerosene, gun powder, iodine, aqua regia, buttermilk, cream and marshmallows. Additionally, innumerable botanicals, such as snake root, coffee, gelisium,

hellebore, ipecac, lobelia, mustard, opium, strychnine, veratrum, etc., have been suggested.

There are also many known and proposed anti-puritic agents such as calamine lotion, petrolatum and steroids for use to relieve itching and inflammation. Others are disclosed in U.S. Pat. Nos. 4,210,633 (film-forming formulation containing an anti-inflammatory steroid), 4,522,807 (substantive topical composition containing anti-inflammatory steroid), 4,711,780 (itch relieving cream), 4,883,813 (keto- and furyl-butyrolactones), 4,923,900 (composition containing benzoyl peroxide particles), and 4,963,591 (substantive, film-forming cellulosic polymer/solvent system containing an active). Anti-inflammatory compositions employing substituted salicylamide are disclosed in U.S. Pat.Nos. 4,725,590, 4,742,083 and 4,560,549. Anti-irritants which block or eliminate responses to irritant chemicals are disclosed in U.S. Pat. Nos. 4,401,663 (sulfonamide derivatives), 4,424,205 (hydroxyphenylacetamides), 4,443,473 (carbamate derivatives) and 4,460,602 (urea derivatives).

Alternatively, the detoxification of urushiol and like compounds has been suggested wherein these allergens are degraded or contacted with a material capable of reacting therewith to produce non-allergenic reaction products. See U.S. Pat. Nos. 4,002,737 (enzyme degradation), 4,259,318 (enzyme degradation), and 4,594,239 (chlorine-containing compound capable of reacting with urushiol).

Barriers and protectants meant to keep the allergen off the skin have been proposed. U.S. Pat. No. 3,749,772 discloses a composition based upon a film-forming acrylic polymer base which is crosslinked by a complexed metal as a linking agent upon application to the skin, thereby forming a selective membrane thereon. Though the film is water-insoluble, the film is alcohol and perspiration soluble. U.S. Pat. No. 4,663,151 discloses the topical application of

aluminum chlorhydrate as a prophylactic treatment for poison oak, poison ivy and poison sumac. U.S. Pat. No. 4,861,584 discloses an allergen absorbent and blocking composition containing an organo-treated clay of the smectite type which is also topically applied to the skin.

U.S. Pat. Nos. 3,961,044, 3,981,990, 4,045,550, 4,076,799, 4,137,301, 4,141,966, 4,144,319 and 4,160,819 disclose compositions for protecting the skin from dermatologic irritation and methods of inhibiting or reducing skin irritation caused by contact with natural and synthetic allergenic agents. These compositions incorporate a protective agent which is generalized as an organic compound containing at least two polar groups which are separated by a chain of at least 15 atoms a majority of which are carbon atoms and preferably containing a cyclic moiety of at least 5 atoms. A variety of polar groups are identified as suitable compounds fitting the foregoing generalized description thereof.

While the art has provided various methods and compositions for preventing and relieving allergic contact dermatitis, the need still exists for a method and composition for preventing the skin irritation or dermatitis caused by natural and synthetic allergenic compounds. For example, present methods are generally directed to the relieving of symptoms of allergenic contact dermatitis, rather than preventing such dermatitis. Present barriers or protectants have not been commercially accepted and/or are not as effective, long-lasting and cosmetically acceptable as presently desired by the public. These barriers or protectants also tend not to be substantive to the skin and/or not to be film-forming.

Cationically charged aminopolysaccharides are known to be substantive and film-forming. Various compositions containing these materials are disclosed in U.S. Pat. Nos. 4,979,722 (sunscreen skin cremes, moisturizing cremes and

wound dressings) and 4,946,870 (delivery systems for actives). Neither of these two patents relate to a method for preventing or minimizing skin contact with materials which produce allergic contact dermatitis.

Accordingly, it was surprising to discover that these cationically charged aminopolysaccharides when topically applied to the skin in a pharmaceutically acceptable carrier could also prevent or minimize skin contact with materials which produce allergic contact dermatitis.

SUMMARY OF THE INVENTION

Accordingly, one or more of the following objects will be achieved by the practice of the present invention.

It is an object of this invention to reduce the amount of skin irritation due to allergic contact dermatitis caused by contact with an allergen following sensitization to that allergen.

Another more specific object of the present invention is to prevent or reduce skin irritation resulting from contact of the skin with irritating plants of the genus Rhus.

Another more specific object of the present invention is to prevent or minimize contact of the skin with irritating plants of the genus Rhus.

Another more specific object of the present invention is to prevent or reduce skin irritation resulting from contact of the skin with allergenic and/or toxic agents formed or used in industrial processes.

Another more specific object of the present invention is to prevent or minimize contact of the skin with allergenic and/or toxic agents formed or used in industrial processes.

It is a still further object of this invention to provide a method wherein compositions containing an aminopolysaccharide are applied to the skin to prevent or reduce dermatologic reaction of the skin due to contact with skin irritating allergenic agents.

It is a still further object of this invention to provide a method wherein compositions containing an aminopolysaccharide and an anionic polymer as an immobilizer for the aminopolysaccharide are applied sequentially to the skin to prevent or reduce dermatologic reaction of the skin due to contact with skin irritating allergenic agents.

It is a still further object of this invention to provide a method wherein compositions containing an aminopolysaccharide are applied to the skin to prevent or reduce skin contact with toxic agents.

It is a still further object of this invention to provide a method wherein compositions containing an aminopolysaccharide and an anionic polymer as an immobilizer for the aminopolysaccharide are applied sequentially to the skin to prevent or reduce skin contact with a toxic agent.

It is still a further object of this invention to provide a skin-protective composition.

Another more specific object is to provide a protective composition capable of screening against toxic agents and/or poison ivy and the like.

Another more specific object is to provide a composition which is effective for protecting skin from the effects of contact with poison ivy and the like.

Another more specific object of this invention to provide a composition wherein the composition comprises an aminopolysaccharide and an anionic polymer as an immobilizer for the aminopolysaccharide which are applied sequentially to the skin to prevent or reduce skin contact with toxic agents and/or to prevent or reduce dermatologic reaction of the skin due to contact with skin irritating allergenic agents.

These and other objects will readily become apparent to those skilled in the art in light of the teachings herein set forth.

Accordingly, the present invention is directed to a method of protecting the skin from contact with an allergenic

agent comprising applying to the skin of a subject sensitized to said allergenic agent, prior to contact with said skin irritating allergenic agent, a biocompatible, substantive, film-forming protective composition, said protective composition comprising at least one aminopolysaccharide, said irritation of the skin being an allergenic contact dermatitis, said skin irritating allergenic agents being allergic contact dermatitis producing agents and said at least one aminopolysaccharide being present in an amount effective to reduce skin irritation compared to skin irritation produced in the absence of said at least one aminopolysaccharide. Preferably, the at least one aminopolysaccharide is selected from the group consisting of chitosonium polymers and covalent chitosan derivatives. The protective composition preferably further comprises a non-toxic pharmacologically acceptable base or carrier, wherein said at least one aminopolysaccharide is dissolved or dispersed in said base or carrier.

Preferably, the method further comprises applying an immobilization composition over said applied protective composition, said immobilization composition comprising at least one anionic compound, preferably an anionic polymer, said at least one anionic compound being present in an amount effective to render said at least one aminopolysaccharide water-insoluble. Likewise, the immobilization composition preferably further comprises a non-toxic pharmacologically acceptable base or carrier, wherein said at least one anionic compound is dissolved or dispersed in said base or carrier, which may be the same or different from that of the protective composition.

The invention further relates to a skin protective composition for protecting the skin from contact with a toxic agent or a skin irritating allergenic agent prepared by first applying a first composition to the skin of a subject, prior to contact with said toxic and/or skin irritating allergenic

agent, said first composition comprising at least one aminopolysaccharide, and then applying a second composition over said applied first composition, said second composition comprising at least one anionic compound, preferably an anionic polymer, said at least one anionic compound being present in an amount effective to render said at least one aminopolysaccharide water-insoluble, said irritation of the skin being an allergenic contact dermatitis, said skin irritating allergenic agents being allergic contact dermatitis producing agents and said at least one aminopolysaccharide being present in an amount effective to reduce skin irritation compared to skin irritation produced in the absence of said at least one aminopolysaccharide or said at least one aminopolysaccharide being present in an amount effective to prevent or at least minimize contact of said toxic agent with the skin of the subject compared to skin contact of said toxic agent in the absence of said at least one aminopolysaccharide, respectively.

The second composition may, optionally, further contain an insect repellent and/or UV absorber or sunscreen. These materials are effectively blocked from contacting the skin by the first composition. Furthermore, these materials are also immobilized by the composite combination of the first and second composition. The insect repellent would be slowly released from the second composition in a repellent effective amount.

DETAILED DESCRIPTION OF THE INVENTION

As has been previously indicated, the invention of the present invention relates to the discovery that aminopolysaccharides when applied to the skin prevent, or at least minimize, contact of the skin with natural and synthetic toxins and/or allergens, such as the natural allergens produced by poison ivy, poison oak and poison sumac. Examples of such aminopolysaccharides and methods for

their manufacture are disclosed in U.S. Pat. Nos. 4,929,722 and 4,946,870, the disclosures of which are hereby incorporated in their entirety.

There are several features which make the skin protective compositions of the present invention superior barriers and protectants. In the first instance, the skin protective compositions used in the method of this invention are substantive with skin and the mucous membrane of human beings. The term "substantive" as used throughout the specification indicates a cohesive interaction between the aminopolysaccharide and a proteinaceous substrate. Substantivity is obtained either by having a cationic charge on the polymer which can be obtained by protonation or quaternization, or by incorporation of appropriate hydrophobic groups or combinations thereof. Thus, the skin protective compositions of the present invention exhibit a cohesive interaction with the proteins of the skin and mucosa of a human being.

Also, those aminopolysaccharides which are cationically charged exhibit substantive properties to keratin and other protein constituents of skin and mucosa. Thus, upon application of, for example, a cationic chitosan derivative to these tissues, the resulting film is strongly bound to the tissue, also inhibiting the loss or migration of the film produced thereby.

The aminopolysaccharides, particularly the chitosan derivatives, are good film formers. When one of these derivatives is topically applied to the skin or mucosa in the form of a lotion, solution, creme, ointment, spray, aerosol, powder, and the like, a polymer film readily forms thereon which acts as a protectant or barrier to allergenic agents, thereby protecting the skin of a subject who is sensitized to such allergenic agents. Alternatively, the aminopolysaccharide, for example a chitosan derivative, may also be applied to the skin or mucosa in the form of a

pre-formed film, sponge, powder or other composite. An additional feature is that the chitosan derivatives which are free of naturally associated proteins, heavy metals and the like are biocompatible and non-irritating to living tissue. They also fail to elicit an inflammatory allergic or pyrogenic response in humans after ingestion or percutaneous or subcutaneous application. In addition, these chitosan derivatives form films on skin and mucosa that are imperceptible to the subject and cosmetically comfortable to wear. The chitosan derivatives are also good humectants.

Preferably, the aminopolysaccharides employed in the compositions and methods of this invention are chitosonium polymers and covalent chitosan derivatives. The chitosonium polymers are soluble in water and in mixtures of water and alcohol, and readily form humectant films, and are also substantive to skin and mucosa. These chitosonium polymers may be prepared by a number of methods including direct dissolution, spray drying, lyophilization, and the acid decrystallization process described in U.S Pat. Nos. 4,929,722 and 4,946,870, previously incorporated herein by reference.

However, the route of preparation of the chitosonium polymers is not critical to this invention. Examples of the chitosonium polymers include those wherein one or more of the amino groups thereof have been neutralized by acids, which may include, but not limited to,: pyrrolidone carboxylic, acetic, lactic, glycolic, glyceric, mandelic, salicylic, benzoic, itaconic, malic, nicotinic, glutamic and aspartic acids, and the acid form of other amino acids such as N-acetyl methionine, N-acetyl tyrosine, N-acetyl glycine, N-benzoyl serine, and the like.

The second type of aminopolysaccharide preferably employed in the compositions and methods of this invention are covalent chitosan derivatives. These derivatives are prepared by the reaction of chitosan with one or more

electrophilic reagents such as ethylene oxide, propylene oxide, glycidol, alkyl halides (from C1 to C24), glycidyl trialkylammonium salts (alkyl groups from C1 to C24), 3-chloro-2-hydroxypropyl ammonium salts, 1,3-propanesultone, haloacetates, succinic anhydride, maleic anhydride, acyl halides, the N-carboxy-alpha-amino acid anhydrides, and the like. These chitosan derivatives are readily soluble in either water, alcohol, water/alcohol mixtures, or other organic solvents such as ether, acetone, or ethyl acetate. These derivatives are good film formers, good humectants, and are substantive if cationic and/or hydrophobic groups are included in the polymer backbone.

Certain of the aminopolysaccharides which can be employed in the skin protective compositions of the present invention include those prepared by an acid decrystallization method as set forth in U.S. Pat. No. 4,929,722. These aminopolysaccharides can be conveniently prepared by a method which comprises the steps of:

(a) forming a mixture of

(1) a pulverulent, partially deacetylated aminopolysaccharide and

(2) a diluent medium in which the aminopolysaccharide is swellable but essentially insoluble; the medium comprised of:

(i) an inert, water soluble, polar organic diluent in which the aminopolysaccharide is insoluble and the aminopolysaccharide derivative is insoluble;

(ii) at least one organic acid which is at least partially soluble in water, is sufficiently acidic to form the ammonium salt of the aminopolysaccharide and yet not sufficiently acidic to cause hydrolysis of the aminopolysaccharide or derivative, and which is present in an amount sufficient to

protonate the reactive sites of the deacetylated aminopolysaccharide; and

(iii) water in an amount up to about 45 weight percent of said medium;

(b) agitating the mixture at a temperature and for a period of time to effect at least partial decrystallization; and

(c) recovering the aminopolysaccharide derivative from the mixture.

As previously indicated, a variety of derivatives of aminopolysaccharides, such as chitosan, can be prepared. These derivatives can be ionic compositions (salts) or covalent compositions.

To prepare covalent chitosan derivatives such as esters, amides and ethers,the swollen, decrystallized slurry of the chitosan salt (also referred to herein as chitosonium polymer or salt) prepared by the aforementioned method, is causticized with a stoichiometric excess of a base such as sodium hydroxide and then reacted with various electrophiles, such as ethylene oxide, glycidol, 1,2-epoxy dodecane, chloroacetic acid, succinic anhydride, and the like.

To prepare ionic derivatives in the form of salts of chitosan, the acid used in the decrystallization step is chosen to provide the desired functional group and both decrystallization and derivatization, i.e. salt formation, is accomplished simultaneously. Alternatively, as indicated above, the organic acid utilized in the decrystallization step can be selected so that the chitosan is not only decrystallized but the salt is obtained containing the desired organic function present in the acid employed.

As indicated above, certain of the aminopolysaccharides are prepared by the above-referenced, heterogeneous method for the decrystallization of aminopolysaccharides and to a variety of derivatives having properties which render them

particularly attractive for use as a protectant or barrier to allergenic agents.

The acid decyrstallization method differs from the methods disclosed in the literature in several respects. First, the acid decrystallization process does not involve dissolving the aminopolysaccharide, such as chitosan, in an aqueous medium. Since chitosan is a very rigid molecule, only a very limited quantity can be rendered water soluble before the solution becomes too viscous to be easily handled. If the solution is further diluted to overcome the viscosity problem, the concentration of chitosan is reduced even further and hence any chemical reactions to derivatize the molecule are very inefficient and economically unattractive.

For example, in literature currently available by a company engaged in the commercial sale of chitosan in the United States, it is indicated that chitosan is soluble in solutions of most acids, particularly organic acids such as formic acid, malic, tartaric, citric, adipic, and the like. It is further indicated that in order to make a one percent solution of chitosan in water, chitosan is mixed with water and then an equal volume of an acid solution is added. For concentrated solutions of chitosan, which are indicated in the literature reference to be from about 2 to 4 percent by weight, an equal weight of acid to that of the chitosan is employed. With inorganic acids such as hydrochloric or nitric acids chitosan is soluble within the range of 0.15 to 1.1 percent acid by weight. Chitosan is not soluble in sulfuric acid and has only marginal solubility in phosphoric acid at concentrations below 0.5 percent.

Thus, prior to the above-referenced method of decrystallization, no method was reported in the literature whereby aminopolysaccharides could be decrystallized and derivatized in economically attractive quantities by a simple and efficient process.

A variety of acids can be used in the decrystallization process. It is, of course, necessary that the acid be at least partially soluble in water, be sufficiently acidic to form the ammonium salt of the aminopolysaccharide and yet not sufficiently acidic to cause hydrolysis of the aminopolysaccharide or derivative, and which is present in an amount sufficient to protonate the reactive sites of the deacetylated aminopolysaccharides.

Such acids can be represented by the formula:

$$R-(COOH)n$$

wherein n has a value of 1 or 2 and R represents a mono- or divalent organic radical composed of carbon, hydrogen and optionally at least one of oxygen, nitrogen and sulfur. Preferred acids are the mono- and dicarboxylic acids composed of carbon, hydrogen, oxygen and nitrogen, and which are at least partially water soluble, and biologically and/or pharmaceutically acceptable for use in the skin protective compositions and methods of the present invention.

Accordingly, a wide variety of acids can be employed which not only effect decrystallization of chitosan, but simultaneously afford desirable derivatives as well. Illustrative acids, in addition to those previously mentioned include, among others, formic, acetic, N-acetylglycine, acetylsalicylic, fumaric, gallic, glycolic, iminodiacetic, itaconic, DL-lactic, maleic, DL-malic, methacrylic, 2-pyrrolidone-5-carboxylic, salicylic, succinamic, succinic, ascorbic, aspartic, adipic, glutamic, glutaric, malonic, nicotinic, pyruvic, sulfonyldiacetic, thiodiacetic and thioglycolic acids.

As indicated above, the medium employed in the decrystallization of the chitosan is a combination of water and an organic compound. This diluent system which is employed in the decrystallization process is a combination of water and an organic compound. Organic compounds which are useful are those which are water soluble, in which the

aminopolysaccharide is insoluble, and in which the aminopolysaccharide derivative is insoluble. Illustrative organic compound which can be employed include acetone, methanol, ethanol, n-propanol, isopropanol, tertiary butyl alcohol, acetonitrile, tetrahydrofuran, dioxane, 2-ethoxyethanol, dimethoxyethane, and the like.

The second component of the diluent medium is water and it is employed in an amount up to about 45 weight percent of the total medium, i.e., the total of the water plus the organic compound. In practice, optimum results are obtained when the diluent medium contains from about 30 to about 45 weight percent water and more preferably about 40 weight percent.

In contrast to other methods in the prior art for preparing such materials, this method avoids formation of a chitosan solution. By this process, the chitosan is caused to swell and accordingly viscous solutions containing only a few percent of chitosan are avoided.

The sequence of mixing the diluent medium and the deacetylated chitosan is not necessarily critical. However, it has been observed that excellent results are obtained if the diluent medium is prepared from the water and organic compound together with the acid and then the chitosan added.

As previously indicated, chitosan has a very rigid structure and when it dissolves in acid solution it provides a very viscous product of low concentration of chitosan. In order for chitosan to be soluble at all, it must have a relatively large number of free primary amine groups. The chitosan employed in the present invention is deacetylated chitin and the degree of deacetylation is normally in excess of 50 percent, preferably in excess of 60 percent and more preferably in excess of 70 percent. The molecular weight range of the chitosan employed in the present invention preferably ranges from about 10,000 to over ten million and more preferably from about 10,000 to about 10,000,000.

Particularly preferred is chitosan having a molecular weight of from about 20,000 to about 2,000,000. A one (1) percent by weight solution of the aminopolysaccharide hydrated in cold water preferably yields a solution viscosity from about 5 to about 5,000 centipoise, more preferably from about 5 to 3,000 centipoise, using a Brookfield viscometer model LVT, spindle #2 at 6 rpm.

Thus, using acids of the aforementioned formula, the method can be employed in the preparation of a variety of salt derivatives of chitosan having utility as indicated above. For example, the pyrrolidone carboxylic acid (PCA) derivative of chitosan is an effective moisturizing agent, has a low order of irritation and accordingly is useful in skin protective compositions of the present invention. As indicated in U.S. Pat. No. 4,929,722, such a polymer is prepared by reacting a finely ground slurry of chitosan with PCA in a polar solvent such as aqueous ethanol, or other suitable solvent that will dissolve PCA. Chitosonium pyrrolidone carboxylate has a large number of other useful applications such as topical medical formulations. While chitosan accelerates healing, the PCA is a built-in humectant.

Any number of other chitosan salt derivatives may be made by the method of U.S. 4,929,722. This method for preparing chitosan salts is applicable to other organic acids that are soluble in polar organic solvents such as ethanol. For example, glycolic acid in aqueous ethanol can be reacted with chitosan to give the glycolate salt, which is also useful as a protectant or barrier.

Moreover, the healing properties of chitin and chitosan have been reported. In addition to being effective fungicides, these polysaccharides are reportedly useful in accelerating the healing rate of wounds or of any irritation related eruptions that may occur. For example, chitosonium lactate has been used as a burn covering. A solution is

sprayed on the burn, forming a covering to protect the injury, while being permeable to oxygen and speeding the healing of the burn. For typical applications requiring a water-soluble form of chitosan, chitosonium lactate may be employed.

When free of its naturally associated proteins, chitin is not antigenic to human tissue and may be used on, or inserted under the skin, or placed in contact with body fluids without harm. Chitin in the body is slowly attacked by lysozyme and is absorbed. In addition chitin and chitosan may be safely ingested by humans, for example, common foods such as bread, beer, wine, shrimp, crabs and mushrooms all contain some chitin.

Glycosaminoglycans (GAGS) are a class of polysaccharides that occur in the connective tissue of mammals, and include hyaluronic acid, chondroitin sulfate, and heparin. Some of these polysaccharides, hyaluronic acid in particular, have been used successfully for wound healing and tissue regeneration in both humans and laboratory animals. The exact mechanism of tissue regeneration is not known, but oligomeric metabolites of N-acetylglucosamines and glucosamine functionality present in glycosaminoglycans such as hyaluronic acid is present in chitin and chitosan, and similar wound healing and tissue regeneration properties have been reported for chitin and chitosan.

Moreover, it has been reported in the literature that growth inhibition of Pseudomonas aeruginosa, Staphylococcus aureus, and Staphylococcus epidermis on agar plates were noted with 1 % chitosan solutions in dilute acetic acid. Parallel experiments with the fungus Candida tropicalis and chitosan solutions also exhibited fungal growth inhibition. Similar results were reported on the fungistatic action of chitosan on plant pathogens.

In addition to the chitosonium polymers and covalent chitosan derivatives prepared as described above, the skin

protective compositions of the present invention can be comprised of chitosonium polymers and covalent chitosan derivatives prepared by known methods.

Chitosonium polymers suitable for use in the skin protective compositions and methods of the present invention include salts of chitosan prepared with the following acids:

Acetic
N-Acetyl-L-cysteine
N-Acetyl glycine
Acetylsalicylic
Adipic
L-Aspartic
Citric
Fumaric
2-Furoic
Gallic
L-Glutamic
Glutaric
Glycolic
Hydrochloric
4-Hydroxybenzoic
Iminodiacetic
Itaconic
3-Ketoglutaric
DL-Lactic
Maleic
DL-Malic
Malonic
Nicotinic (Niacin)
2,3-Pyridinedicarboxylic
2-Pyrrolidone-5-carboxylic
Pyruvic
Salicylic
Succinamic
Succinic
Sulfanilic
Sulfonyldiacetic
L-Tartaric
Thioacetic
Thiolactic
Vanillic

Combinations of these acids also are suitable.

These salts are readily soluble in water at room temperature, except for the malate, maleate, itaconate, salicylate, fumarate, and succinate salts, which require heating to about 75°C to effect dissolution, after which

they remain soluble. The products from the reaction of acrylic, citric, gallic, 4-hydroxybenzoic, and vanillic acids, when used alone, are only slightly soluble in water, because the reaction by which the derivative is formed has limited efficiency.

Illustrative chitosonium polymers which can be prepared by the above process include, but are not limited to, chitosonium pyrrolidone carboxylate, chitosonium itaconate, chitosonium niacinate, chitosonium salicylate, chitosonium lactate, chitosonium formate, chitosonium acetate, chitosonium fumarate, chitosonium gallate, chitosonium glutamate, chitosonium maleate, chitosonium succinamate, chitosonium aspartate, chitosonium glycolate and the like.

Once applied to the skin, the aminopolysaccharide(s), which are salts thereof or are cationically charged and topically applied to the skin, are then preferably immobilized to provide a water-resistant film at the application site essentially without diminishing the substantivity of the film and with the added benefit of further enhancing the barrier quality of the aminopolysaccharide with respect to allergenic and toxic agents. The immobilized film is resistant to dissolution in water, perspiration and other aqueous environments. The immobilization is preferably accomplished by applying an anionic compound onto the already topically applied cationically charged aminopolysaccharide(s), thereby creating a complex between the two materials, or by raising the pH thereof to change the aminopolysaccharide salt to its water-insoluble form minus the anionic salt moiety thereof.

The preferred cationically charged aminopolysaccharides are the chitosonium polymers which are salts of chitosan. Such chitosonium polymers, which include, but are not limited to, chitosonium salicylate, chitosonium lactate and chitosonium pyrrolidone carboxylate, are soluble in aqueous

solutions at pH less than about 5.5. However, the anionic salt moiety is displaced if the pH exceeds about 5.5, and the polymer is changed from soluble chitosonium salt to an insoluble form of chitosan. This insoluble chitosan forms a film which is resistant to dissolution in water. This water-insoluble film may appear to be a gel or a high viscosity solution.

Various methods of forming the water-insoluble film by increasing the pH of the topically applied cationically charged aminopolysaccharide, e.g. chitosonium salt, may be utilized. For example, a chitosonium polymer-containing composition is applied to the desired topical site. Then, a quantity of an inorganic base, for example sodium bicarbonate and sodium borate (borax), (typically in an aqueous solution) sufficient to increase the pH to at least about 5.5 is applied to the site over the applied chitosonium polymer-containing composition. The higher pH causes the formation of the water-insoluble film.

Alternatively, the chitosonium polymer-containing composition may further include a compound which will dissociate the anionic moiety from the chitosonium salt to form the insoluble layer at the time the skin protective composition is applied to the skin. For example, an amine carbonate can be incorporated into the skin protective composition containing the chitosonium salt. At the pH of this composition (typically between about 4.5 and 5.5) before application to the skin, the carbon dioxide would remain associated with the amine. However, when the skin protective composition is applied, the pH of the substrate (skin pH is about 4.5) causes the evolution of carbon dioxide, which frees the amine moiety to react with the chitosonium salt, displace the anion, and produce the water-insoluble chitosan film. Examples of such amines include carbonates of mono-, di- and tri-ethanol amines and related compounds.

Immobilized films of this type are "water-proof"' as defined by federal authorities in relation to sunscreens. Without the immobilization, a high molecular weight chitosonium salt, i.e., molecular weight greater than about 100,000, as part of a skin protective composition would be considered "water-resistant", as defined by federal authorities in relation to sunscreens.

Alternatively, cationically charged aminopolysaccharides can be rendered water-insoluble by complexing them with an anionically charged compound, preferably a polyanionically charged compound and more preferably a polyanionically charged polymer so as to form a polyion interpolymer complex and effectively crosslinking the aminopolysaccharide which is polycationically charged. For example, natural or synthetic polycarboxylic acid salts may be used. These salts are preferably obtained by neutralizing the corresponding polycarboxylic acid with an inorganic base such as borax (sodium borate), sodium bicarbonate, sodium carbonate, sodium hydroxide and ammonium hydroxide.

Natural polycarboxylic acids include, but are not limited to, alginic acid and glycosaminoglycans. Glycosaminoglycans in their free acid and salt form are disclosed in U.S. Pat. No. 4,767,463 and 4,913,743, the disclosures of which are incorporated herein by reference.

Synthetic polycarboxylic acids are preferably polymers including homopolymers, copolymers and graft polymers. The homopolymer is a polymer of an ethylenically unsaturated mono-carboxylic acid or an ethylenically unsaturated poly-carboxylic acid or cyclic anhydride thereof. The copolymer may be a copolymer of an ethylenically unsaturated mono-carboxylic acid and/or an ethylenically unsaturated poly-carboxylic acid or cyclic anhydride thereof copolymerized with, optionally, one or more ethylenically unsaturated non-carboxylic acid-containing monomers. The graft polymer

is a homopolymer or copolymer of at least one ethylenically unsaturated, non-carboxylic acid-containing monomer to which is grafted an ethylenically unsaturated mono-carboxylic acid or an ethylenically unsaturated poly-carboxylic acid or cyclic anhydride thereof. The homopolymer and copolymer may be the product of polymerizing one or more conjugated-dienes, such as butadiene or isoprene, which may then optionally be selectively hydrogenated leaving a residual amount of ethylenic unsaturation therein at which ethylenically unsaturated carboxylic acids may be grafted. Generally, polymerization and hydrogenation are carried out in solution with a suitable catalyst therefor. The grafting reaction may take place in solution or in the melt, such as in an extruder. Such polymerization, copolymerization and grafting processes and methods are well-known to those skilled in the art, as well as selective hydrogenation. Examples of such materials are disclosed in U. S. Pat. Nos. 3,749,772, 4,374,126 and 4,522,807, the disclosures of which are incorporated herein by reference.

The ethylenically unsaturated carboxylic acids utilized in the polymers hereof preferably have 2 to about 10 carbon atoms excluding those in the carboxyl and/or cyclic anhydride groups thereof.

Examples of such ethylenically unsaturated mono--carboxylic acids include, but are not limited to, acrylic acid, methacrylic acid, crotonic acid, and the like. Optionally, oligomers or polymer sequences of these monomers may be capable of forming a cyclic anhydride which may be utilized to incorporate hydrophobic moieties into the polymer or cationically-charged moieties into the polymer.

The ethylenically unsaturated poly-carboxylic acids and cyclic anhydrides thereof are preferably ethylenically unsaturated dicarboxylic acids and cyclic anhydrides

thereof and more preferably alpha, beta-ethylenically unsaturated dicarboxylic acids and cyclic anhydrides thereof.

Examples of such ethylenically unsaturated poly--carboxylic acids and cyclic anhydrides thereof include, but are not limited to, maleic acid, fumaric acid, maleic anhydride, itaconic acid, itaconic anhydride, citraconic acid, mesaconic acid, citraconic anhydride, aconitic acid (a tricarboxylic acid), aconitic anhydride, cis-4-cyclohexene-1,2-dicarboxylic acid, cis-4-cyclohexene-1,2-dicarboxylic anhydride, endo-cis-bicyclo (2,2,1)-5-heptene-2,3- dicarboxylic acid, and endo-cis-bicylco (2,2,1)-5-heptene-2,3- dicarboxylic anhydride. These modifiers may be used alone or in combination thereof. Among these ethylenically unsaturated poly-carboxylic acids and cyclic anhydrides thereof, maleic acid, fumaric acid and maleic anhydride are particularly preferred, with maleic anhydride most preferred.

Examples of ethylenically unsaturated non-carboxylic monomers include, but are not limited to, vinyl ethers, vinyl esters, vinyl amides, and olefins.

Examples of vinyl ethers include, but are not limited to, vinyl methyl ether, vinyl dodecyl ether, divinyl ether, and vinyl isopropyl ether.

Examples of vinyl esters include, but are not limited to, vinyl acetate, vinyl stearate, and vinyl laurate.

Examples of vinyl amides include, but are not limited to, N-vinyl pyrrolidone.

Examples of olefins include, but are not limited to, ethylene, propylene, styrene, acrylonitrile, vinyl imidazole, vinyl pyridine and conjugated-dienes, for example, butadiene and isoprene.

Additionally, these copolymers may be block, tapered, random or regularly alternating copolymers. Again such copolymerization processes and methods and resulting

polymers are well-known to those skilled in the art. Examples of such block copolymers are HYPAN (Reg. TM) copolymers available from Kingston Technologies of Dayton, New Jersey, which include block copolymers of acrylic acid and acrylonitrile according to U.S. Patent No. 4,420,589. Examples of such regularly alternating polymers are UCARSET (Reg. TM) polymers available from Union Carbide Co., which include regularly alternating polymers of vinyl methyl ether and maleic anhydride. Examples of poly(acrylic acid) polymers are CARBOMER or CARBOPOL (Reg. TM) polymers available from B.F. Goodrich Chemical Group, Cleveland, Ohio.

As earlier indicated, there is a growing concern regarding the systemic absorption through the skin of materials which are presently in common use as insect repellents such as DEET (N,N-diethyl-m-toluamide) and UV absorbers or sunscreens such as benzophenone, PABA (para-aminobenzoic acid) and esters thereof and substituted PABA, esters of para-methoxycinnamic acid, benzotriazoles and aminophenols. Once the aminopolysaccharide-containing composition has been topically applied to the skin, insect repellents and/or UV absorbers or sunscreens may be applied thereon and effectively blocked thereby from contacting the skin. Preferably, the aminopolysaccharide(s) have been immobilized prior to their application to thereby enhance the barrier properties of the aminopolysaccharide(s) thereon to such materials. Alternatively, these insect repellents and/or UV absorbers or sunscreens are more preferably incorporated into the immobilizing composition, thereby incorporating them into the substantive film on the skin yet blocked from contacting the skin by the aminopolysaccharide layer of the film. In this manner, these materials are more tightly bound in place and resistant to removal by contact with water and/or perspiration through normal activity.

The aminopolysaccharide-containing composition may also contain and be used for the delivery of pharmaceutical or therapeutic actives such as disclosed in U.S. Pat. No. 4,946,870, earlier incorporated herein by reference. Such actives may be incorporated in the compositions utilized herein so long as such actives do not detract from the barrier properties of the aminopolysaccharide with respect to the allergenic agent(s), i.e., that the active(s) does not provide the allergenic agent(s) an avenue to the skin or enhance the penetration thereof through the aminopolysaccharide layer to the skin. The same conditions and requirements would apply to penetration and/or delivery enhancers which would be used to aid in the delivery of the active(s).

In general, the amount of aminopolysaccharide(s) employed in the skin protective compositions of this invention will vary depending upon the presence or absence of pharmaceutical or therapeutic actives and, if present, the particular pharmaceutical or therapeutic actives, the presence or absence of a diluent, the type of other additives, and the like. In practice, however, it has been found that a concentration of the aminopolysaccharide in the composition can range from about 0.5 to about 20 weight percent, more preferably about 3 to about 8 weight percent, based on the total weight of the composition.

If desired, the skin protective compositions and the immobilization compositions used in this invention in addition to the aminopolysaccharide and the anionic compound, respectively, can contain one or more pharmaceutically acceptable diluents or vehicles. In many instances, the aminopolysaccharide(s) and the anionic compound(s) themselves can be about 0.5 to about 30 weight percent of their respective compositions with the remainder

being a diluent or vehicle and, optionally, other pharmaceutically acceptable additives. Suitable diluents include among others, water, ethanol, aqueous ethanol, isopropanol, glycerine, dimethylether, carbon dioxide, butane, polyethylene glycol, ethoxylated or propoxylated glucose, sorbitol derivatives, and the like.

Although the inclusion of the aminopolysaccharide(s) in the skin protective compositions of this invention usually avoids the necessity for using ointments, oils and other aesthetically undesirable carriers, in some instances it may be helpful to include such compounds.

In practice, the skin protective compositions and immobilization compositions of the invention are readily formulated. Other pharmaceutically acceptable ingredients include adjuvant ingredients and humectants such as glycerine, propylene glycol, and sorbitol; preservatives; stearic acid; cetyl alcohol and other high molecular weight alcohols; surfactants and dispersants such as polysorbate 20 and PPG-12-buteth-16; silicone polymers such as dimethicone copolyol; menthol, eucalyptus oil and other essential oils, fragrances, and the like; skin protectants such as allantoin and zinc stearate; auxiliary gellants such as hydroxymethyl cellulose and xanthum gum; to give stable creams, ointments, lotions, aerosols, solutions, may also be included in the respective compositions.

An embodiment of the skin protective composition may be formulated in the form of a lotion having the following ingredients and proportions:

	COMPOSITION	
Ingredients	(%w/w);	(Preferred)
Purified water	60 - 80	(60-70)
chitosonium lactate	1-10	(3-8)

1,3-butylene glycol	1-15	
Alcohol SD-40	5-10	(7-9)
PPG-12-buteth-16	1-5	(1-3)
Dimethicone copolyol	2-6	(3-5)
Allantoin	0.05 - 1	(0.5-1)
Zinc acetate	0.10-0.15	
Hydroxyethylcellulose	0.01-1.0	(0.05-0.75)
Polysorbate 20	0.02-1.0	

An embodiment of the immobilization composition may be formulated in the form of a lotion having the following ingredients and proportions:

	COMPOSITION	
Ingredients	(%w/w)	(Preferred)
Purified water	50-70	(70-85)
Carbomer (polyacrylic acid)	0.01-0.5	(0.1-0.3)
Xanthum gum	0.01-0.5	(0.025-0.075)
Alcohol SD-40	1-20	(8-15)
PPG-12-buteth-16	1-5	
Dimethicone copolyol	0.01-5	(0.1-3)
Sodium borate (borax)	0.01-1	(0.1-0.5)
Polysorbate 20	0.01-1	

The following examples are intended to further illustrate the invention, not to limit it in any way. The scope of the invention is limited only to the scope of the appended claims.

Examples 1 through 15 are directed to the preparation of compositions which would be useful as a skin protective composition in the method of the present invention and which have additionally identified utilities. These examples are identified with their other stated utility. Examples 4 and 5 are directed to immobilized aminopolysaccharide films.

Examples 16 and 17 are directed to an in vitro assessment made of the barrier properties of the aminopolysaccharide film using chitosonium lactate as the

aminopolysaccharide. The allergenic agent utilized was a CARDOLITE (Reg. TM) material, an industrial lubricant which is like urushiol without the hydroxyl moiety at the 2-position.

Example 18 is directed to an in vivo test performed to determine the effectiveness of chitosonium lactate when challenged by urushiol on a sample of human subjects in a double-blind fashion.

The skin protective compositions used in the examples were prepared using aminopolysaccharides in the form of chitosonium polymers prepared by the acid decrystallization method as well as known derivatives prepared by methods disclosed in the literature. Unless otherwise indicated the solution viscosity of the chitosonium polymers is between about 5 and 5,000 centipoise (cP) at 1% aqueous solution and 20°C, as measured using a Brookfield viscometer model LVT, spindle #2 at 6 rpm.

EXAMPLES

Throughout the Examples, all parts are parts by weight, unless otherwise identified.

EXAMPLE 1

CHITOSONIUM SALICYLATE SUNSCREEN SKIN CREAM

Chitosonium salicylate (1.0 g, prepared as described in example 5 of U.S. Patent No. 4,929,722) was mixed with 78.0 g of distilled water, and heated to 60°C. until all of the polymer had dissolved. Propylene glycol (10.0 g) was added, with stirring at 60°C. Separately, a solution of cetyl alcohol (6.0 g), stearic acid (3.0 g), silicone oil 7002 (1.0 g, from Union Carbide), and TWEEN 20 (1.0 g, from ICI, Inc.) was prepared at 60°C., and while stirring vigorously, this solution was added to the aqueous chitosonium salicylate solution at 60°C. After stirring for five

minutes, the agitation was stopped, and the mixture was allowed to cool to give a white skin cream. The UV absorbance spectrum of chitosonium salicylate exhibits a maximum at 298 nm (epsilon = 3.6×10^3 M^{-1} cm^{-1}), which is in the center of the UV-B region (320-290 nm). Therefore, chitosonium salicylate is an effective sunscreen agent.

EXAMPLE 2

PREPARATION OF CHITOSONIUM PYRROLIDONE CARBOXYLATE MOISTURIZING CREAM

Chitosonium pyrrolidone carboxylate (1.0g, prepared as described in Example 3 of U.S. Patent 4,929,722, previously incorporated herein by reference) was dissolved in 78.0 g of distilled water. The solution was heated to 60°C. and 10.0 g of propylene glycol was added with stirring. Separately, a solution of cetyl alcohol (6.0 g), stearic acid (3.0 g), silicone oil 7002 (1.0 g, from Union Carbide), and BRIJ 98 (1.0 g, from ICI, Inc.) was prepared at 60°C. and while vigorously stirring, this solution was added to the aqueous chitosonium pyrrolidone carboxylate solution at 60°C. After stirring for five minutes, the agitation was stopped, and the mixture was allowed to cool to give a white, moisturizing skin creme, to condition skin and accelerate healing of damaged skin.

EXAMPLE 3

PREPARATION OF CHITOSONIUM PYRROLIDONE CARBOXYLATE-HYALURONIC ACID BLENDS AS WOUND DRESSINGS

A solution (50 g) of 0.1% (by weight) commercial, extraction grade sodium hyaluronate was ion-exchanged by stirring with 5 g of AMBERLITE 200 ion exchange resin for 1 hour. The resin was removed by vacuum filtration. A solution (45 ml) of 1% (by weight) chitosonium pyrrolidone carboxylate in distilled water was place in a 100 ml Waring blender, and stirred at a rate of 3000 rpm. While

vigorously mixing the chitosonium pyrrolidone carboxylate solution, 45 ml of ion-exchanged sodium hyaluronate were added by syringe over one minute and the mixture was stirred at 3000 rpm for two minutes. The product was a highly viscous, very slightly milky, homogeneous blend of chitosonium pyrrolidone carboxylate and hyaluronic acid which is useful as a wound dressing or coating to protect tissues and accelerate healing.

If the two polymer solutions are mixed in reverse order (chitosonium pyrrolidone carboxylate added to hyaluronic acid) or the stirring rate is insufficient to give a good mixing, the chitosonium pyrrolidone carboxylate/hyaluronic acid blend is rendered insoluble and precipitates.

The procedure above was repeated with commercial, fermentation grade sodium hyaluronate with the same results. In both cases, the ion exchange step may be omitted, but the resulting homogeneous chitosonium pyrrolidone carboxylate/sodium hyaluronate blends are somewhat more opaque.

EXAMPLE 4

USE OF AQUEOUS CHITOSONIUM POLYMERS IN TWO COMPONENT SPRAYABLE WOUND DRESSINGS

A solution of chitosonium pyrrolidone carboxylate was prepared by dissolving 2.0 g of chitosan (low molecular weight, 1% solution viscosity in 1% aqueous acetic acid of less than 100 cP at 30 rpm) and 1.2 g of 2-pyrrolidone-5-carboxylic acid in 98 g of sterile water. Alternatively, 3.2 g of solid chitosonium pyrrolidone carboxylate; prepared by spray drying, freeze drying, the method of U.S. Pat. No. 4,929,722, or other appropriate method, can be dissolved directly in 98 g of sterile water. The polymer solution was then charged to a plastic pump spray bottle (A). A second solution of sodium alginate (Sigma Chemical Company) was prepared by dissolving 0.33 g of sodium

alginate in 99 g of sterile water, and this solution was charged to a second plastic pump spray bottle (B).

Clean glass plates heated to 37°C. were used as the substrate to model a typical dermal surface. While warmed at 37°C., solution A was sprayed on and allowed to dry for a few minutes, then solution B was sprayed on and also allowed to dry for a few minutes. A control experiment was conducted in which only solution A was sprayed on the substrate, and solution B was omitted. The plates were then immersed in distilled water at 25°C. and allowed to stand for 30 minutes, to simulate the effect of body fluid on the dressing.

The plate coated only with solution A (control) exhibited complete dissolution of the film from the surface of the plate, demonstrating loss of the film dressing in water. However, the plate coated with both solutions A and B gave a polyelectrolyte composite film which retained adhesion to the surface of the plate even when totally immersed in water. This behavior of adhesion in water is highly desirable in a wound dressing. The compatibility of chitosonium polymers with tissue is well documented. Although this example uses chitosonium pyrrolidone carboxylate, other chitosonium salts such as itaconate, ascorbate, nicotinate, lactate, acetate, glutamate, and aspartate would be equally suitable. Other suitable anionic polymers such as natural anionic polymers including sodium hyaluronate, chondroitin sulfate, keratin sulfate, carrageenan, heparin, and carboxymethylcellulose can be used instead of sodium alginate.

EXAMPLE 5

PREPARATION AND OXIDATIVE CROSS-LINKING OF CHITOSONIUM ASCORBATE

A 250 ml, three-necked round bottomed flask was fitted with a stirring paddle and motor, and two rubber serum

caps. The flask was charged with 6.0 g of commercial chitosan (ground to pass a 0.5 mm screen) and 60 ml of isopropyl alcohol, and the flask was fitted with a subsurface nitrogen feed (syringe needle) and a mineral oil bubbler as an outlet. Because of the oxidative sensitivity of the product, the reaction and the work-up were conducted under nitrogen, and all dissolved oxygen was removed from all solutions and diluents by purging with nitrogen for 30 minutes or longer.

While stirring the slurry under nitrogen for 1 hour, a solution of 6.62 g of L-ascorbic acid (Aldrich Chemical Company) in 25 ml of distilled water was prepared. This solution was then added to the chitosan slurry by syringe, and after 30 minutes, a solution of 26 ml of isopropyl alcohol and 11 ml of distilled water was added.

After stirring two additional hours, the flask was transferred to a GLOVE-BAG (Reg. TM) (I^2R), and the polymer was recovered by vacuum-filtration under nitrogen. The polymer was washed under nitrogen once with a solution of 160 ml of isopropyl alcohol and 40 ml of water, and once with 200 ml of isopropyl alcohol. The light tan solid was briefly dried under nitrogen, and a 2% solution of the polymer in deoxygenated water was prepared. Films were cast on tin plates at ambient temperature using this solution: some films were allowed to dry in air, while others were dried under nitrogen. The films dried under nitrogen remained colorless and were readily soluble in water, while the films dried in air exhibited a yellowish tint and were insoluble in water, even on prolonged heating in water. Although the mechanism of insolubilization is not well understood, it is presumed to be oxidative because of the importance of oxygen being present during film insolubilization. Such a room temperature, air curing polymer system would have utility in personal care and biomedical applications such as wound dressings, skin care,

pharmaceutical delivery systems, slow-release for fragrance, cosmetic, or medicament delivery, among others.

EXAMPLE 6

PREPARATION OF A CHITOSAN-BASED BURN TREATMENT LOTION

0.15 g of ethyl 4-aminobenzoate (benzocaine) was dissolved in 3.85 g of ethylalcohol and 1.0 g of water. 5.0 g of 2.0% aqueous chitosonium pyrrolidone carboxylate was added, and after vigorous mixing, a clear, colorless solution was obtained. This lotion is useful in the treatment of sunburns and other minor burns. The benzocaine (1.5%) is a local anesthetic which would alleviate pain and discomfort, and chitosonium pyrrolidone carboxylate is an excellent humectant which moisturizes the skin.

EXAMPLE 7

PREPARATION OF CHITOSAN-BASED CORTICOSTEROID LOTION

0.013 g of hydrocortisone was dissolved in 4.99 g of ethyl alcohol, and mixed with 5.0 g of 2% aqueous chitosonium pyrrolidone carboxylate, giving a clear, colorless solution. This solution (0.13% hydrocortisone) is useful in the topical treatment of a variety of local inflammatory diseases and pruritus. Substituting 0.015 g of dexamethasone for 0.013 g of hydrocortisone in this formulation yields a clear, colorless solution of 0.15% dexamethasone, a fluorinated steroid, also used in the treatment of topical inflammatory diseases and general inflammation.

EXAMPLE 8

PREPARATION OF A CHITOSAN-BASED NON-STEROID ANTI-INFLAMMATORY LOTION

0.50 g of ibuprofen was dissolved in 4.5 g of ethyl alcohol, and mixed with 5.0 g of 2% chitosonium niacinate in 90:10 water/alcohol, giving a clear, colorless solution (5.0% ibuprofen). This solution is useful for a variety of localized inflammations, including topical treatment of muscle pain, as well as tendon, ligament, and muscle sprains.

EXAMPLE 9

PREPARATION OF CHITOSAN-BASED ANTIHISTAMINE LOTION

0.025 g of chlorpheniramine maleate was dissolved in 0.05 g of glycerine and 9.925 g of 1.5% aqueous chitosonium pyrrolidone carboxylate, giving a clear, colorless solution (0.25% chlorpheniramine maleate). This lotion is useful in the treatment of rashes and inflammation due to allergic reactions.

EXAMPLE 10

PREPARATION OF A CHITOSAN-BASED CORTICOSTEROID LOTION

0.0225 g of triamcinolone acetonide was dissolved in 4.99 g of ethyl alcohol and mixed with 5.09 g of 2% aqueous chitosonium lactate, giving a clear, colorless solution. This solution (0.225% triamcinolone acetonide) is useful in the topical treatment of a variety of local inflammatory diseases.

EXAMPLE 11-15

PREPARATION OF TOPICAL FORMULATIONS USING COVALENT CHITOSAN DERIVATIVES

Each of the above formulations in Examples 6 through 10 could be repeated with the exception that a covalent chitosan derivative, such as aqueous glycidyl

trimethylammonium chloride/glycidol modified chitosan as described in European Patent No. 0 115 574 and aqueous glycidyl trimethylammonium chloride/propylene glycol modified chitosan, may be substituted for the aqueous chitosonium polymer thereof. These formulations would also be suitable as the protective compositions used in the present invention.

EXAMPLE 16

IN VITRO SYNTHETIC SKIN PERMEATION

A nine(9)-mm inside diameter flat type modified Franz diffusion cell with an open top cap was used for the synthetic skin permeation study. Isopropyl myristate maintained at 25°C. was used as the receptor fluid to simulate the lipid sea in the stratum corneum. A membrane of cellulose acetate/nitrate to simulate human skin was carefully mounted on the top of the cell. CARDOLITE (Reg. TM) NC-511, which is 3-(n-penta-8-decenyl) phenol and is used as a industrial lubricant, is a known allergenic agent and was used herein to model urushiol. A known amount of the CARDOLITE NC-511 phenol (about 50 microliters) was applied onto the membrane surface at the center of the cell covering the entire cell, then the cap thereof was secured in place with a clamp. About two (2) milliliter alliquots of the receptor fluid were drawn at the following time intervals; 1, 2, 5, 7, 10, 15 and 20 minutes. The withdrawn volume of receptor fluid was replenished with fresh isopropyl myristate to maintain the initial volume. The CARDOLITE NC-511 alkylated phenolic resin in the sample solutions was quantified by capillary GC analysis. The membrane specimen was examined visually to ensure that no leakage occurred during the experiment.

The uncoated membrane was used as a control. The foregoing procedure was repeated with coated membranes. The membranes were coated with six different coatings. The

thicknesses of the coatings were 8 mils thick prior to drying and about 0.2 mil thick after drying. Membrane A was coated with a 1% aqueous solution of chitosonium lactate and allowed to dry. Membrane B was coated with a 3% aqueous solution of chitosonium lactate and allowed to dry. Membrane C was first coated with a 3% aqueous solution of chitosonium lactate and allowed to dry; and then immobilized with a sodium bicarbonate spray with water as the carrier therefor and also allowed to dry. Membrane D was first coated with a 3% aqueous solution of chitosonium lactate and allowed to dry; and then immobilized with a poly(acrylic acid sodium salt) with a dilute aqueous alcohol mixture (90/10 water/ethanol) as the carrier therefor and also allowed to dry. The poly(acrylic acid sodium salt) was obtained by neutralizing a poly(acrylic acid) (CARBOPOL 943P having a molecular weight of greater than three million) with borax. Membrane E was first coated with a 6% aqueous solution of chitosonium lactate and allowed to dry; and then immobilized with a poly(acrylic acid sodium salt) with a dilute aqueous alcohol mixture (90/10 water/ethanol) as the carrier therefor and also allowed to dry. This poly(acrylic acid sodium salt) was obtained by neutralizing a poly(acrylic acid) (CARBOPOL 934P) with borax. Membrane F was first coated with a 6% aqueous solution of chitosonium lactate and allowed to dry; and then immobilized with a poly(acrylic acid sodium salt) with a dilute aqueous alcohol mixture (90/10 water/ethanol) as the carrier therefor and also allowed to dry. This poly(acrylic acid sodium salt) was obtained by neutralizing a poly(acrylic acid) (CARBOPOL 934P) with sodium hydroxide. The chitosonium lactate utilized in this example had a solution viscosity of about 10-30 centipoise and thus characterized as having a low molecular weight. The results of the permeation study are shown in Table 1.

TABLE 1

TIME	% CARDOLITE in the Receiver						
(min.)	Control	A	B	C	D	E	F
1	0.124	0.02	0	0	0	0	0
2	0.58	0.11	0	0	0	0	0
5	0.33	0.22	0	0.0205	0	0	0
7	0.23	0.26	0.0212	0.0398	0	0	0
10	0.14	0.33	0.0245	0.0688	0.0127	0	0
15	0.13	0.28	0.0328	0.12	0.0147	0	0
20	-----	0.09	0.0457	0.119	0.0092	0	0

The foregoing shows that the aminopolysaccharide film is an effective barrier to the CARDOLITE material, particularly when the aminopolysaccharide is immobilized.

EXAMPLE 17

IN VITRO PERMEATION, MOLECULAR WEIGHT EFFECTS

In this example, the molecular weight of the chitosonium polymer on its barrier properties is investigated. The procedures of Example 16 were repeated using membrane G which like membrane A except that a chitosonium lactate having a solution viscosity of about 100-300 centipoise was used instead. The higher viscosity characterizes this material as having a higher molecular weight than that used in membrane A. The results are tabulated in Table 2.

TABLE 2

TIME (min.)	% CARDOLITE in the Receiver Membrane: Control	A	G
1	0.124	0.02	0.08
2	0.58	0.11	0.265
5	0.33	0.22	0.08
7	0.23	0.26	0.0376
10	0.14	0.33	0.04
15	0.13	0.28	0.133
20	--	0.09	0.153

The foregoing shows that molecular weight has a modest effect on the barrier properties of the aminopolysaccharide film.

EXAMPLE 18

IN VIVO EVALUATION OF SKIN PROTECTIVE COMPOSITION AND METHOD

To evaluate the protective effect against experimental poison oak/ivy dermatitis, an embodiment of the skin protective compositions useful in the method of the present invention was compared to the vehicle thereof as a placebo in a double blind in vivo study.

The skin protective composition (composition X) investigated and utilized for the study was a 6% chitosonium lactate lotion having the composition set out in Table 3. The placebo (composition Y) was the vehicle of composition X.

TABLE 3

Ingredients	COMPOSITION X (%w/w)	Y (%w/w)
Purified water	67.05	74.99
Butylene glycol/chitosonium lactate slurry[a]	20.00	---
1,3-butylene glycol	---	12.00
Alcohol SD-40	8.00	8.00
PPG-12-buteth-16	3.00	3.00
Dimethicone copolyol	1.20	1.20
Allantoin	0.50	0.50
Zinc acetate	0.11	0.11
Hydroxyethylcellulose	0.10	0.10
Polysorbate 20	0.02	0.05
Noville #28927	0.02	0.05

a. slurry contains:

	%w/w
1,3-butylene glycol	70
chitosan lactate	30

(wherein the chitosonium lactate is like that used in Example 16)

The tests were carried out with five (5) subjects known to be very sensitive to poison oak/ivy. These individuals were pretreated on the right and left forearm, in a predetermined randomized fashion, with liberal amounts of the coded materials (compositions X and Y (placebo)) (the code and the randomization sheet were maintained by the technician who made the applications). Four hours later the subjects returned and were tested within the treated sites (outlined by marking ink), with 5 microliters of 3 or 4 dilutions of purified urushiol in acetone (concentrations within the range of 0.25-0.001 mg/ml). These test sites were examined in 2,4,6/7 days and scored on a scale from 0-4 in which:

0 (or N) = no reaction;

1= erythema and edema, involving more than half the test area;

2= erythema, edema and small vesicles involving the full test area;

3= erythema, edema and large vesiculations; and

4= bullae.

Positive reactions that affected less than half the test site were scored as -1, and questionable reactions, usually seen at the first observation, were scored +/-. This procedure of randomized treatments and patch testing was repeated twice in all subjects.

The results are tabulated in Table 4. Chitosan lactate (composition X) was applied most frequently for comparison (8 times) and the placebo (composition Y) was applied 6 times. The chitosan lactate was directly compared to placebo 4 times. The results are displayed in Table 4 Thus, it can be seen that preparation X (chitosan lactate) was better than the control preparation on 3 occasions and less effective on 1 occasion when directly compared. Thus, the placebo (composition Y) was less effective in protecting against the rash in 3 out of 4 direct comparisons against composition X and the chitosonium lactate gave more protection than the placebo.

In this study there was no evidence of irritation from any of the preparations used, and at four hours after application it was generally not possible to see any material on the skin sites that were tested. Thus, chitosonium lactate did not appear to be irritating.

Therefore, chitosonium lactate as a 6% concentration in a liquid vehicle appeared more effective than the base as a barrier preparation against experimental poison oak/ivy dermatitis in four direct comparisons utilizing 5 subjects and a randomized, double blind in vivo method.

TABLE 4

Subject	Trial	Composition	Day	Concentration of Urushiol (mg/ml) 0.25	0.1	0.05	0.025	0.01	0.005	0.0025	0.001
1	1	X	2	—	—	1	N	N	—	—	—
			4	—	—	2	N	N	—	—	—
			6	—	—	2[a]	N	N	—	—	—
	1	Y	2	—	—	2	N	N	—	—	—
			4	—	—	2-3	±	N	—	—	—
			6	—	—	3-4	N	N	—	—	—
	2	X	2	—	—	N	N	N	—	—	—
			4	—	—	1	N	N	—	—	—
			6	—	—	Heal	N	N	—	—	—
2	1	Y	2	—	—	—	N	N	N	N	—
			4	—	—	—	2	-1	N	N	—
			6	—	—	—	2	±	N	N	—
	1	X	2	—	—	—	N	±	N	N	—
			4	—	—	—	1	-1	N	N	—
			6	—	—	—	1	-1	N	N	—
	2	X	2	—	—	—	N	N	N	—	—
			4	—	—	—	N	N	N	—	—
			6	—	—	—	N	N	N	—	—
3	1	Y	2	—	—	—	—	—	N	1	N
			4	—	—	—	—	—	N	1-2	N
			6	—	—	—	—	—	N	Heal	N
	2	X	2	—	—	—	—	—	N	-1	N
			4	—	—	—	—	—	N	1	N
			6	—	—	—	—	—	N	Heal	N

a. healed

TABLE 4 (Continued)

Subject	Trial	Composition	Day	Concentration of Urushiol (mg/ml) 0.25	0.1	0.05	0.025	0.01	0.005	0.0025	0.001
4	1	X	2	—	—	—	1	N	N	—	—
			4	—	—	—	2	1	N	—	—
			6	—	—	—	2	1-2	N	—	—
	2	Y	2	—	—	—	-1	N	N	—	—
			4	—	—	—	1	N	N	—	—
			6	—	—	—	1	N	N	—	—
	2	X	2	—	—	—	±	N	N	—	—
			4	—	—	—	2	-1	N	—	—
			6	—	—	—	2	1	N	—	—
5	1	X	2	N	N	N	N	—	—	—	—
			4	1	N	N	N	—	—	—	—
			6	1	N	N	N	—	—	—	—
	1	Y	2	1	N	N	N	—	—	—	—
			4	2	N	N	N	—	—	—	—
			6	2	N	N	N	—	—	—	—
	2	Y	2	1	N	N	—	—	—	—	—
			4	2	N	N	—	—	—	—	—
			6	2	N	N	—	—	—	—	—

The foregoing in vitro and in vivo studies have clearly shown that the aminopolysaccharides utilized in the compositions and methods of the present invention are effective in the prevention or minimization of allergenic agent contact with the skin. The barrier properties of the aminopolysaccharide either alone or in conjunction with an immobilizer should also be effective in preventing or minimizing contact of toxic agents with the skin.

The foregoing in vitro and in vivo studies have clearly shown that the aminopolysaccharides utilized in the compositions and methods of the present invention are effective in the prevention or minimization of allergenic agent contact with the skin. The barrier properties of the aminopolysaccharide either alone or in conjunction with an immobilizer should also be effective in preventing or minimizing contact of toxic agents with the skin.

It will be apparent from the foregoing that many other variations and modifications may be made in the methods and the compositions herein before described, by those having experience in this technology, without departing from the concept of the present invention. Accordingly, it should be clearly understood that the methods and the compositions referred to herein in the foregoing description are illustrative only and are not to have any limitations on the scope of the invention as set out by the appended claims.

WHAT IS CLAIMED IS:

1. A method of protecting the skin from contact with an allergenic agent comprising applying to the skin of a subject sensitized to said allergenic agent, prior to contact with said skin irritating allergenic agent, a biocompatible, substantive, film-forming protective composition, said protective composition comprising at least one aminopolysaccharide, said irritation of the skin being an allergenic contact dermatitis, said skin irritating allergenic agents being allergic contact dermatitis producing agents and said at least one aminopolysaccharide being present in an amount effective to reduce skin irritation compared to skin irritation produced in the absence of said at least one aminopolysaccharide.

2. The method of claim 1, wherein said at least one aminopolysaccharide is selected from the group consisting of chitosonium polymers and covalent chitosan derivatives.

3. The method of claim 1, wherein said protective composition further comprises a non-toxic pharmacologically acceptable base or carrier therefor, wherein said at least one aminopolysaccharide is dissolved or dispersed in said base or carrier.

4. The method of claim 1, said method further comprising applying an immobilization composition over said applied protective composition, said immobilization composition comprising at least one anionic compound, said at least one anionic compound being present in an amount effective to render said at least one aminopolysaccharide water-insoluble.

5. The method of claim 4, wherein said at least one anionic compound is an anionic polymer.

6. The method of claim 4, wherein said immobilization composition further comprises a non-toxic pharmacologically

acceptable base or carrier therefor, wherein said at least one anionic compound is dissolved or dispersed in said base or carrier.

7. A method of protecting the skin from contact with an toxic agent comprising applying to the skin of a subject, prior to contact with said skin irritating toxic agent, a biocompatible, substantive, film-forming protective composition, said protective composition comprising at least one aminopolysaccharide, said at least one aminopolysaccharide being present in an amount effective to prevent or at least minimize contact of said toxic agent with the skin of the subject compared to skin contact of said toxic agent in the absence of said at least one aminopolysaccharide.

8. The method of claim 7, wherein said at least one aminopolysaccharide is selected from the group consisting of chitosonium polymers and covalent chitosan derivatives.

9. The method of claim 7, wherein said protective composition further comprises a non-toxic pharmacologically acceptable base or carrier therefor, wherein said at least one aminopolysaccharide is dissolved or dispersed in said base or carrier.

10. The method of claim 7, said method further comprising applying an immobilization composition over said applied protective composition, said immobilization composition comprising at least one anionic compound, said at least one anionic compound being present in an amount effective to render said at least one aminopolysaccharide water-insoluble.

11. The method of claim 10, wherein said at least one anionic compound is an anionic polymer.

12. The method of claim 10, wherein said immobilization composition further comprises a non-toxic pharmacologically acceptable base or carrier therefor, wherein said at least one anionic compound is dissolved or dispersed in said base

or carrier.

13. A skin protective composition for protecting the skin from contact with a skin irritating allergenic agent or a toxic agent, said protective composition being prepared by first

applying a first composition to the skin of a subject, prior to contact with said skin irritating allergenic agent or said toxic agent, said first composition comprising at least one aminopolysaccharide, and

then applying a second composition over said applied first composition,

said second composition comprising at least one anionic compound and at least one ingredient selected from the group consisting of an insect repellent, UV absorber and a combination thereof,

said at least one anionic compound being present in an amount effective to render said at least one aminopolysaccharide water-insoluble,

said irritation of the skin being an allergenic contact dermatitis,

said skin irritating allergenic agents being allergic contact dermatitis producing agents, and

said at least one aminopolysaccharide being present in an amount effective to reduce skin irritation compared to skin irritation produced in the absence of said at least one aminopolysaccharide or said at least one aminopolysaccharide being present in an amount effective to prevent or at least minimize contact of said toxic agent with the skin of the subject compared to skin contact of said toxic agent in the absence of said at least one aminopolysaccharide, respectively.

14. The composition of claim 13, wherein said anionic compound is an anionic polymer.

15. The composition of claim 13, wherein said at least one aminopolysaccharide is selected from the group consisting

of chitosonium polymers and covalent chitosan derivatives.

16. The composition of claim 13, wherein said first composition further comprises a non-toxic pharmacologically acceptable base or carrier therefor, wherein said at least one aminopolysaccharide is dissolved or dispersed in said base or carrier.

17. The composition of claim 13, wherein said second composition further comprises a non-toxic pharmacologically acceptable base or carrier therefor, wherein said at least one anionic compound is dissolved or dispersed in said base or carrier.

INTERNATIONAL SEARCH REPORT

International Application No. PCT/US91/08914

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all)[3]

According to International Patent Classification (IPC) or to both National Classification and IPC

IPC (5): C08B 37/08; A61K 9/02; 7/135 A61K 37/48, A61K 7/34, A61K 7/38
US CL : 514/55, 514/602; 424/94; 424/47; 514/63

II. FIELDS SEARCHED

Minimum Documentation Searched[4]

Classification System	Classification Symbols
U.S.	514/55, 514/602; 424/94; 424/47; 514/63

Documentation Searched other than Minimum Documentation to the extent that such Documents are included in the Fields Searched[5]

APS

III. DOCUMENTS CONSIDERED TO BE RELEVANT[14]

Category*	Citation of Document,[16] with indication, where appropriate, of the relevant passages[17]	Relevant to Claim No.[18]
Y	US,A, 4,929,722 (Partain, III et al.) 29 May 1990, See abstract, columns 21 (lines 10-68) and 22 (lines 8-66).	13-17
Y	US,A, 4,946,870 (Partain, III et al.) 7 August 1990, see entire document.	1-12
Y	US,A, 4,594,239 (Pluim, Jr.) 10 June 1986, see columns 1 (lines 5-31) and 2 (lines 25-54).	13-17
&	US,A, 4,767,463 (Brode et al) 30 August 1988, see columns 1, 2 & 3.	13-17
&	US,A, 4,913,743 (Brode et al) 30 April 1990, see columns 1, 2 & 3.	13-17
A	US,A, 4,002,737 (Borris) 11 January 1977. See entire document.	1-17
A	US,A, 4,010,252 (Hewitt) 01 March 1977, see entire document	1-17
Y	US,A, 4,668,666 (Allan et al.) 26 May 1987.	13-17
A	US,A, 4,137,301 (willer et al) 01 January 1979. See entire document.	1-17

* Special categories of cited documents:[15]

"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier document but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search[2]	Date of Mailing of this International Search Report[2]
27 FEBRUARY 1992	16 MAR 1992
International Searching Authority[1]	Signature of Authorized Officer[20]
ISA/US	Louise Leary te

Form PCT/ISA/210 (second sheet)(May 1986) ß